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Task Force

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Document Control Office (7407)
Office of Pollution Prevention and Toxics
Environmental Protection Agency
Room G-099
401 M Street, S.W.
Washington, D.C. 20460

Contains No CBI

Re: OPPTS-42187B; FRL-4869-1

Dear Sirs:

On June 26, 1996, EPA proposed a test rule for 21 hazardous air pollutants (HAPs), including 1,1,2-trichloroethane and ethylene dichloride (EDC), and solicited proposals for enforceable consent agreements (ECAs) regarding the performance of pharmacokinetics studies for particular HAPs to permit extrapolation from oral data to inhalation exposure. 61 Fed. Reg. 33177. On October 18, EPA extended the deadlines for submission of proposals for ECAs for pharmacokinetics studies to November 25, 1996. 61 Fed. Reg. 54383.

The HAP Task Force represents manufacturers of 1,1,2-trichloroethane and EDC. The Task Force has retained ChemRisk to develop ECA proposals for these substances. Three copies of each proposal are enclosed.

The HAP Task Force appreciates EPA's willingness to extend the deadline for these proposals and for comments on the proposed test rule. As a result of this extension, we were able to have a productive meeting with EPA scientific staff on November 5, which also facilitated the development of proposals which we believe will provide EPA the information it requires more quickly and efficiently than would a test rule, while avoiding unnecessary animal testing.

Please do not hesitate to let me know if there is any question about these proposals.

Sincerely,

Peter E. Voytek

Peter E. Voytek, Ph.D.
Manager

Enclosures

cc: Mr. Gary E. Timm
Michael L. Gargas, Ph.D.
W. Caffey Norman, Esq.

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PROPOSAL FOR PHARMACOKINETICS STUDY OF ETHYLENE DICHLORIDE

November 22, 1996

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Prepared by:

ChemRisk® --A Division of McLaren/Hart
Courtland East Building
29225 Chagrin Boulevard
Cleveland, OH 44122

PROPOSAL FOR PHARMACOKINETICS STUDY OF ETHYLENE DICHLORIDE

1.0 INTRODUCTION

On June 26, 1996, the USEPA proposed a test rule under Section 4(a) of the Toxic Substances Control Act (TSCA) which would require manufacturers and processors of 21 hazardous air pollutants, including ethylene dichloride (EDC), to test these compounds for specific health effects. The tests proposed to be conducted for EDC via inhalation exposures include the following:

- acute toxicity,
- subchronic toxicity,
- developmental toxicity,
- reproductive toxicity, and
- neurotoxicity.

The cost for conducting these tests was estimated by the USEPA to be approximately $2 million. As an alternative to conducting these tests, the USEPA is soliciting proposals regarding the use of pharmacokinetic studies which would permit the extrapolation of toxicity information from other routes of exposure (*i.e.*, oral) to predict risks from inhalation exposures. This document serves as a proposal to conduct pharmacokinetic studies for EDC as a means of filling specific data gaps.

Prior to extrapolating information from one route to another, an evaluation of available information must be made with respect to its adequacy for route-to-route extrapolation. A decision tree for

conducting route-to-route extrapolations has been proposed (Gerrity and Henry, 1990), and is provided in Figure 1. Following an evaluation of the information available, there are four possible options that can be pursued:

Option 1: Collect data (all routes)

Option 2: Use data for a structurally analogous compound

Option 3: Collect data for relevant routes (inhalation)

Option 4: Route-to-route extrapolation

Route-to-route extrapolation may be conducted using various levels of complexity ranging from the use of default absorption values to physiologically based pharmacokinetics (PBPK) modeling. The approach proposed here centers on the use of a validated PBPK model.

Three general requirements are necessary to conduct proper route-to-route extrapolations using physiologically based pharmacokinetic (PBPK) models:

1. A scientifically plausible (defensible) mechanism of toxicological action is needed.

2. Studies must be available that are considered adequate to assess relevant toxicological endpoints via a route(s) of exposure other than that of interest.

3. A validated PBPK model for the chemical and species of interest must be available which is capable of predicting the pertinent internal dose measures based on mechanism of action.

When these criteria exist for a compound that also has no toxicological effects on the portal of entry, a route-to-route extrapolation can be performed as follows:

1. The internal dose metric is determined under the experimental conditions for the studies to be extrapolated (*i.e.* oral) for the NOAEL and/or LOAEL doses.

2. Equivalent NOAEL and/or LOAEL concentrations for route extrapolated to (*i.e.* inhalation) are determined by estimating an exposure that produces the same internal dose metric as determined in Step 1 above.

For EDC, an evaluation was made of primary and secondary toxicological literature to determine the adequacy of the dose-response data for the effects listed above following inhalation, oral, and other relevant exposures. Limited information from inhalation studies for EDC in animals suggests that effects remote from the respiratory tract (*i.e.*, liver, kidney) may be of potential concern. For oral exposures, potential candidate studies were located for route-to-route extrapolation (Option 4) for acute toxicity, subchronic toxicity, neurotoxicity, and reproductive toxicity (only if necessary). A recent inhalation study that evaluated developmental effects appears to fill that data gap (Section 3.4).

The remainder of this proposal will address the status of each of these requirements with regard to EDC, and will describe the specific approaches we propose to use to fill certain data gaps identified in the test rule.

2.0 MECHANISM OF ACTION

EDC is metabolized to compounds [2-chloroacetaldehyde, S(2-chloroethyl)glutathione] that are capable of binding covalently to macromolecules (Fabricant and Chalmers, 1980; Jean and Reed, 1989). EDC has also been shown to promote lipid peroxidation (ATSDR, 1994). Hepatic DNA damage in mice was unaffected by a cytochrome P-450 inhibitor (piperonyl butoxide), but was diminished by the addition of a glutathione depleting agent (diethyl maleate) (Storer and Conolly, 1985). It has been suggested that EDC-induced toxicity occurs when the biotransformation processes (*i.e.*, cytochrome P-450) become saturated, thereby allowing for higher levels of EDC to circulate throughout the body and conjugate with glutathione (activation), instead of being detoxified and eliminated (D'Souza *et al.* 1987; Reitz *et al.* 1982). This mechanism of action is also supported by studies on ethylene dibromide (White *et al.* 1983). It would appear that much of the toxicity associated with EDC exposure could be associated with the formation of glutathione metabolites.

Binding to key cellular molecules by glutathione metabolites of EDC is offered as a potential mechanism of action for the acute toxicity and subchronic toxicity of EDC. Information regarding the potential mechanism(s) of action for EDC-induced neurotoxicity were not located. However, the rapid onset of the anesthetic effects of chlorinated solvents generally precludes the involvement of a metabolite in the mechanism of action (Cassaret and Doull, 1996), and suggests that certain neurological effects of EDC and other solvents are likely associated with the parent compound. Necrosis of the nervous system, however, might be more appropriately associated with glutathione metabolites of EDC.

3.0 EVALUATION OF CANDIDATE STUDIES FOR ROUTE-TO-ROUTE EXTRAPOLATION

3.1 Potential for Direct Contact Effects

An important factor to consider prior to conducting a route-to-route extrapolation is the potential for direct contact (portal of entry) effects on the lung following inhalation exposures. In general, the USEPA has classified inhaled chemicals into one of three categories based on water solubility and reactivity (USEPA, 1994):

Category 1: Do not penetrate to blood (*i.e.*, highly water soluble/very reactive)

Category 2: Water soluble/blood accumulation

Category 3: Water insoluble/perfusion limited

Portal of entry effects do not appear to be of primary concern for EDC based on the following rationale:

- Limited information indicates that effects on the lungs are only of potential concern following acute exposures to very high concentrations in air. Pulmonary congestion was observed in mice, rats, rabbits, and guinea pigs exposed to lethal concentrations of EDC (3,000 ppm) for 7 hours (Heppel *et al.* 1945). This effect was not observed in animals exposed to lower concentrations. In addition, pathological changes of the

lungs were not observed in animals exposed to EDC via inhalation for longer periods (Heppel *et al.* 1946; Cheever *et al.* 1990).

- Preferential accumulation of EDC in the lungs following inhalation exposures does not appear to occur. Rather, EDC rapidly distributes to other tissues following exposure. For example, following inhalation exposure to 50-250 ppm EDC for 2-3 hours, the levels of EDC in the lungs were lower than those observed in the blood and much lower than those observed in adipose tissue (Spreafico *et al.* 1980). The distribution pattern for EDC is very similar for both inhalation and oral exposures (Spreafico *et al.* 1980).

- EDC is described as having a pleasant, sweet odor with an odor threshold of 50-100 ppm (ATSDR, 1994), therefore irritation of the respiratory tract from air concentrations at or below these levels is unlikely.

Based on the low reactivity of EDC in the lung (see above), and the fact that EDC has low water solubility (see saline:air partition coefficient, Gargas *et al.*, 1989), this compound should be considered a Category 3 compound (USEPA, 1994) in which direct effects on the portal of entry are not to be expected.

3.2 Acute Toxicity

A single study was identified as a potential candidate for route-to-route extrapolation for acute toxicity, Daniel *et al.* (1994). This study is summarized briefly below.

- *Daniel et al. (1994)* - Groups of 10 male and 10 female Sprague-Dawley rats were exposed to 0, 10, 30, 100, or 300 mg/kg-day EDC via corn oil gavage for 10 days. Significant mortality (10/10 females, 8/10 males) was noted at the highest dose. Body weight, clinical chemistry, and hematological findings in exposed animals were not significantly different from controls. The main histopathological change noted was inflammation of the forestomach in animals receiving 100 mg/kg-day or more. This endpoint represents a direct contact effect of EDC, which is attributable in part to the mode of administration (*i.e.*, high concentrations administered as a bolus dose in corn oil). Since this effect is not extrapolatable to inhalation exposures, this study identifies a NOAEL of 100 mg/kg-day for histopathological changes in the liver and kidney.

This study is compared with acute toxicity test guidelines from the USEPA (OPPTS) in Table 1. Based on this comparison, this study was considered to be adequate for route-to-route extrapolation. As discussed in Section 3.1, the potential for portal-of-entry effects for EDC on the lungs is low. Based upon the mechanism of action proposed in Section 2.0, the glutathione metabolites of EDC are most likely responsible for any toxic effects. Therefore, it is proposed that some internal measure of these metabolites (*i.e.*, total amount metabolized by the glutathione pathway in a 24 hour period)

be modeled as the appropriate internal dose measure and an equivalent inhalation exposure determined.

3.3 Subchronic Toxicity

Three studies were identified as potential candidates for route-to-route extrapolation for subchronic toxicity, two by NTP (1991) and one by Daniel *et al.* (1994). These studies are summarized briefly below.

- *NTP (1991 - Drinking Water Study in Rats)* - Groups of 10-20 male and 10 female F344/N, Osborne-Mendel, and Sprague-Dawley rats were exposed to drinking water containing 0, 500, 1,000, 2,000, 4,000, or 8,000 ppm EDC for 13 weeks. The actual doses received by the animals varied slightly between species and sex, but were generally between 0, 49-82, 86-126, 147-213, 259-428, 515-727 mg/kg-day for the respective water concentrations. Mortality was not significantly affected by exposure in any strain or sex. The remaining findings from this study are discussed according to rat strain below.

  - *F344/N* - Water intake and body weights were affected in F344/N rats at the two highest doses. No compound-related clinical signs were noted. The authors attributed slight changes in hematological parameters to mild dehydration. Liver and kidney weights were elevated in all exposed animals compared to controls. Histopathological changes in the liver were not observed. While mild renal tubular

regeneration was comparable to controls in exposed male rats, the incidence of this effect was increased in a dose-dependent manner in female rats exposed to 1,000 ppm or more.

- *Sprague-Dawley* - Water intake and body weights were affected in Sprague-Dawley rats at the two highest doses. No compound-related clinical signs were noted. The authors attributed slight changes in hematological parameters to mild dehydration. Liver and kidney weights were elevated in all exposed animals compared to controls. Histopathological changes in the liver were not observed. The incidence of mild renal tubular regeneration was comparable to controls in exposed male and female rats.

- *Osborne-Mendel* - Water intake and body weights were affected in Osborne-Mendel rats exposed to 1,000 ppm (females) or 2,000 ppm (males) or more. No compound-related clinical signs were noted. The authors attributed slight changes in hematological parameters to mild dehydration. Kidney weights were elevated in all dosed females. Liver weights were elevated in exposed males receiving 1,000-2,000 ppm. Histopathological changes in the liver were not observed. The incidence of mild renal tubular regeneration was not significantly different from controls in exposed male and female rats.

This study identifies a NOAEL and LOAEL of 58 and 102 mg/kg-day, respectively for the effects of EDC on the kidney in F344/N rats.

- *NTP (1991 - Corn Oil Gavage Study in Rats)* - Groups of 10-20 male and 10 female F344/N rats were exposed to 0, 30, 60, 120, 240, or 480 mg/kg-day (males), or 0, 18, 37, 75, 150, or 300 mg/kg-day (females) EDC via corn oil gavage for 13 weeks. Significant mortality was noted in male rats (all) receiving 240 mg/kg-day or more, and in female rats (9/10) exposed to 300 mg/kg-day. Body weights were decreased in rats exposed to the highest dose. Liver and kidney weights were elevated in all exposed animals, however histopathological changes in these tissues were not observed. Forestomach effects (hyperplasia, inflammation, and mineralization) were noted in animals that died or were moribund. Necrosis of the thymus was observed in males exposed to 240 mg/kg or more, and in females exposed to 300 mg/kg. The incidence of renal tubular regeneration was comparable between exposed and control animals. Histopathological changes in the liver were not observed. Neurological effects were also observed in animals exposed to the highest doses of EDC (see Section 3.3). This study identifies a LOAEL of 240 mg/kg-day and a NOAEL of 120 mg/kg-day for necrosis of the thymus.

- *NTP (1991 - Drinking Water Study in Mice)* - Groups of 10 male and 10 female B6C3F1 mice were exposed to drinking water containing 0, 500, 1,000, 2,000, 4,000, or 8,000 ppm EDC for 13 weeks. These concentrations corresponded to doses of 0, 249, 448, 781, 2,710, or 4,207 mg/kg-day (males) and 0, 244, 647, 1,182, 2,478, or 4,926 mg/kg-day (females). Significant mortality (9/10) limits the interpretation of results from female mice exposed to the highest dose. All other exposed animals survived the full 13 week exposure. Compound-related clinical signs were not observed in any dose group. Body weights were lower in all

exposed males, and in females exposed to 1,000 ppm or more. Liver and kidney weights were elevated in all exposed animals. In addition, mild-to-moderate tubular regeneration was noted in males exposed to the two highest doses. Histopathological changes in the liver were not observed. This study identifies a NOAEL and LOAEL of 781 and 2,710 mg/kg-day for renal effects in mice.

- *Daniel et al. (1994)* - Groups of 10 male and 10 female Sprague-Dawley rats were exposed to 0, 37.5, 75, and 150 mg/kg-day EDC via corn oil gavage for 90 days. No treatment-related effects were noted regarding mortality, clinical observations, ophthalmology, gross pathology, or histopathology in exposed animals. Body weight gain and food consumption were significantly decreased in male rats exposed to the highest dose. Statistically significant differences in hemoglobin, hematocrit, red blood cell count, platelets, albumin, and alkaline phosphatase were noted in animals exposed to 75 mg/kg-day or more. Organ weight changes (liver, kidney, brain) were also noted in animals exposed to the two highest doses. This study identifies a LOAEL 75 mg/kg-day and NOAEL of 37.5 mg/kg-day for hematological effects and organ weight changes.

These studies are compared with toxicity test guidelines from the USEPA (OPPTS) in Table 2. Based on this comparison, all of these studies were considered adequate for route-to-route extrapolation. It is proposed that the NTP (1991) studies using F344/N rats (drinking water and oil gavage) and B6C3F1 mice (drinking water) be used for extrapolation along with the oil gavage study of Daniel *et al.* (1994) using Sprague Dawley rats. As discussed in Section 3.1 the potential for

portal-of-entry effects of EDC on the lungs is low. Based upon the mechanism of action proposed in Section 2.0, the glutathione metabolites of EDC are most likely responsible for any toxic effects. Therefore, it is proposed that some internal measure of these metabolites (*i.e.*, total amount metabolized by the glutathione pathway in a 24 hour period) be modeled as the appropriate internal dose measure. Equivalent inhalation exposures may be determined, as appropriate, from the NOAELs and LOAELs of the 4 test groups identified above.

3.4 Developmental Toxicity

The USEPA identified developmental toxicity as a data gap for EDC based on an evaluation of the literature available in August, 1995 (USEPA, 1995). However, since the time of that evaluation an inhalation developmental study of EDC has been published. This study is summarized below.

- *Payan et al. (1995)* - Groups of 26 pregnant Sprague-Dawley rats were exposed to either 0, 150, 200, 250, or 300 ppm EDC on days 6 through 21 of gestation. Maternal toxicity (decreased maternal body weight gain, and 2 deaths) was noted in animals exposed to the highest concentration. No treatment-related effects were noted on the number of implantations, resorptions, live fetuses, fetal sex ratio, fetal weight, or malformation incidence. Although the pregnancy rate was significantly lower in animals exposed to 250 ppm, this effect was not observed in animals exposed to the highest dose, and therefore

was not considered to be treatment related. This study identifies a LOAEL and NOAEL of 300 and 250 ppm, respectively, for maternal toxicity.

This study was compared to USEPA (OPPTS) guidelines for developmental toxicity tests in Table 3. Taken with the results of the rabbit and rat studies (Shell Oil 1979 study also cited as Rao *et al.* 1980, Schlacter *et al.* 1979; and Murray *et al.* 1980), reviewed in the support document for the proposed test rule (USEPA, 1995), this confirms that EDC is neither a teratogen nor a developmental toxicant. This study is also summarized in Table 3.

Lane *et al.* (1982) have conducted an oral developmental study as summarized below.

- *Lane et al. (1982)* - Groups of 10-18 pregnant female Swiss ICR mice from F1 and F2 litters from a multigenerational study were exposed to 0, 5, 15, or 50 mg/kg/day EDC via the drinking water on days 1 through 21 of gestation. No treatment-related effects were noted on pup survival or terata in either groups. This study identifies a NOAEL of 50 mg/kg-day EDC for developmental effects.

This study was compared to USEPA (OPPTS) guidelines for developmental toxicity tests (Table 3). The only potential limitation of this study is perhaps in the dose selection since an effect level was not demonstrated. Based on the negative results overall in these inhalation and oral developmental studies, developmental toxicity should not be considered as a data gap for EDC.

3.5 Reproductive Toxicity

The USEPA identified reproductive toxicity as a data gap for EDC based on the limitations found in a single study (Rao *et al.* 1980). Although the support document for the proposed rule cites the limitation in this study as "No-Observed-Adverse-Effect Level (NOAEL) was identified", this statement is in error. Although a LOAEL was not identified, the study clearly identifies a NOAEL, below which reproductive effects were not observed. This study is summarized below.

- Rao *et al.* (1980, also cited as Murray *et al.* 1980, Schlacter *et al.* 1979, and Shell Oil 1979) - In a single generation study, groups of 20-30 male and 20-30 female rats were exposed via inhalation to 0, 25, 75, or 150 ppm EDC for 6 hours/day beginning 60 days prior to mating, and continuing through gestation (F1a and F1b). No treatment related effects were observed on fertility index, pup survival, gestation length, sex ratio, or organ weights in pups from either the F1a or F1b litters. This study identifies a NOAEL of 150 ppm for the reproductive effects of EDC.

This study is compared to USEPA (OPPTS) guidelines for reproductive toxicity in Table 4. The potential limitation of this study is that it was not a multigenerational study. Although this was not a multi-generation study, as recommended by the guidelines, it still is relevant, particularly since EDC is not a bioaccumulative chemical (bioconcentration factor = 2 ATSDR, 1994) and potential human exposures to EDC are expected to be much lower than those tested by Rao *et al.* (1980).

Lane *et al.* (1982) conducted an extensive oral multigeneration study that is summarized below.

- *Lane et al. (1982)* - In a multigeneration study, groups of 10 male and 30 female ICR Swiss mice were exposed to 0, 5, 15, or 50 mg/kg-day EDC via the drinking water. The F0 generation was exposed for 5 weeks prior to mating, whereas the F1 generation was exposed to 11 weeks prior to mating. No treatment-related effects on fertility, gestation, terata, pup weight gain, pup survival, or dominant lethal mutations were observed. This study identifies a NOAEL of 50 mg/kg-day for reproductive effects.

In addition to the negative reproductive studies cited above, histopathological evaluation of reproductive tissues from a subchronic study revealed no effects in the mammary gland, ovary, testes, or uterus of rats exposed to 50 ppm EDC via inhalation for 2 years (Cheever *et al.* 1990). Similarly, histopathological effects of the testes, prostate, tunica vaginalis, uterus, mammary gland, and ovary were not observed in rats exposed orally to 47-95 mg/kg-day or in mice exposed orally to 97-299 mg/kg-day for 78 weeks (NCI, 1978). Based on the weight of evidence from these studies, reproductive effects do not appear to be of primary concern for EDC, and would not appear to warrant further study, although the results of Lane *et al.* (1982) could be extrapolated to inhalation exposures with PBPK modeling.

3.6 Neurotoxicity

Two studies were identified as a potential candidates for route-to-route extrapolation, both of which were conducted by NTP (1991). These studies are summarized briefly below.

- *NTP (1991 - Drinking Water Study)* - Groups of 10-20 male and 10 female F344/N, Osborne-Mendel and Sprague-Dawley rats, and 10 male and 10 female B6C3F1 mice were exposed to drinking water containing 0, 500, 1,000, 2,000, 4,000, or 8,000 ppm EDC for 13 weeks. Significant mortality (9/10) limits the interpretation of results from female mice exposed to the highest dose. All other exposed animals survived the full 13 week exposure. No compound related clinical signs, changes in brain weight, or histological changes of the central nervous system (brain and spinal cord) were observed in any of the exposed animals. Although no LOAELs were identified, this study identifies a NOAEL of 8,000 ppm for EDC (approximately 492 mg/kg-day in rats and 4,207 mg/kg-day in mice) for neurological effects.

- *NTP (1991 - Corn Oil Gavage Study in Rats)* - Groups of 10-20 male and 10 female F344/N rats were exposed to 0, 30, 60, 120, 240, or 480 mg/kg-day (males), or 0, 18, 37, 75, 150, or 300 mg/kg-day (females) EDC via corn oil gavage for 13 weeks. Significant mortality was noted in male rats (all) receiving 240 mg/kg-day or more, and in female rats (9/10) exposed to 300 mg/kg-day. Clinical signs, including tremors, salivation, emaciation, abnormal posture, ruffled fur, and dyspnea were noted in males exposed to 240 mg/kg-day and in females exposed to 300 mg/kg-day. In addition, necrosis of the cerebellum was observed in males

exposed to 240 mg/kg or more, and in females exposed to 300 mg/kg. This study identifies a LOAEL of 240 mg/kg-day and a NOAEL of 120 mg/kg-day for clinical signs of neurotoxicity and necrosis of the cerebellum.

These studies are compared with toxicity test guidelines from the USEPA (OPPTS) in Table 5. Based on this comparison, these studies were considered adequate for route-to-route extrapolation.

The studies conducted by NTP clearly demonstrate a vehicle effect associated with EDC-induced neurotoxicity. In drinking water, no effects were observed in rats that received up to 492 mg/kg-day, or in mice that received up 4,207 mg/kg-day. On the other hand, significant signs of neurotoxicity were noted in rats receiving 240-300 mg/kg-day EDC via corn oil gavage. Differences in the kinetics of uptake of EDC from the gastrointestinal tract will be explored as an initial means of resolving the observed differences in toxicity.

We propose that the parent chemical in the central nervous system is likely responsible for the effects described above (less necrosis of the cerebellum) and that the parent compound in the central nervous system (or proportionately in the blood) be modeled as the appropriate internal dose measure for these studies. For necrosis of the cerebellum, we propose that GSH metabolites are likely involved. Equivalent inhalation exposures will be derived for both of these studies.

4.0 PBPK MODELS FOR EDC

This section summarizes the toxicokinetics of EDC and describes existing and validated PBPK models for this chemical in the rat and mouse.

4.1 Toxicokinetics of EDC

EDC is readily absorbed through the lungs and gastrointestinal tract following exposure (ATSDR, 1994). Absorption of EDC in the gastrointestinal tract is strongly affected by the vehicle in which it is administered. For example, Withey *et al.* (1983) noted that peak blood concentrations for EDC were four times higher and three times faster following administration in water than when administered in oil. Following absorption, EDC distributes to tissues throughout the body, with a higher preference for adipose tissue and tissues with a higher lipid content. Following oral and inhalation exposures, levels of EDC in liver and lung were lower than those in the blood (Spreafico *et al.* 1980). There is little difference between oral and inhalation exposure with respect to tissue distribution (ATSDR, 1994).

EDC undergoes biotransformation via two different pathways. EDC is metabolized by cytochrome P-450 to form chlorhydrin and 2-chloroacetaldehyde. Alternatively, EDC can react with glutathione to form S-(2-chloroethyl)glutathione and a glutathione episulfonium ion. Enzymes involved with EDC metabolism appear to become saturated following exposures of 25 mg/kg-day orally, 150 ppm via inhalation, or when blood levels reach 5-10 μg/mL (D'Souza *et al.* 1988; Reitz *et al.* 1982).

EDC is eliminated from the body primarily via urinary excretion and exhaled breath following either oral or inhalation exposures (Reitz *et al.* 1982; Spreafico *et al.* 1980).

4.2 Existing PBPK Model for EDC

A PBPK model has been developed for EDC in the rat and mouse (D'Souza *et al.* 1987). This is a flow-limited model which includes compartments for the lung, liver, richly perfused tissue (*i.e.*, kidney, spleen), poorly perfused tissue (*i.e.*, muscle, skin), and fat (Figure 2). Partition coefficients were available for EDC in Sprague-Dawley rats, F344 rats, and B6C3F1 mice. In addition, a blood:air partition coefficient was available for EDC in humans. The model accounts for the metabolism of EDC by two competing pathways, (1) cytochrome P-450, and (2) glutathione (GSH). Metabolic rates for these two pathways have been determined by gas uptake measurements (Gargas *et al.* 1986). Metabolism via the former pathway is a saturable enzymatic oxidation, whereas metabolism via the latter pathway is essentially a first order reaction at low exposure concentrations. At higher exposures to EDC, a first order reaction between EDC and GSH did not adequately describe EDC metabolism and underestimated EDC concentrations, possibly due to a depletion of GSH. For this reason, GSH depletion (D'Souza *et al.* 1988) has also been incorporated into the model. The PBPK model for EDC has been validated in the rat and mouse by measuring EDC blood concentration and glutathione (GSH) time course concentrations in tissues following various exposures. These exposures included oral gavage doses of 75 and 150 mg/kg, and inhalation exposures to 150 ppm for 7 hours.

4.3 Proposed Model Refinement

Based on the toxicity endpoints observed for EDC following oral exposures (see Section 3.0), the following tissue compartments will need to be added to the existing PBPK model (Figure 2):

- Kidney
- Thymus
- Central nervous system
- Reproductive tissues (if necessary)
- Embryo/fetus (if necessary)

For these compartments, the partition coefficients will be assumed to be the same as those reported for the liver since no organ-specific information were available. Blood flow and organ volumes will be obtained from available lab animal physiology literature. A summary of the proposed PBPK model for evaluating the results from oral studies discussed in Section 3.0 are provided in Table 6.

An interesting observation is that the toxicity of EDC by the oral route was affected by the vehicle in which it was administered (*i.e.*, oil > water). There are several potential mechanisms by which an oil vehicle can impact the toxicity of EDC:

- *Absorption* - An oil vehicle can change the rate or extent of EDC absorption from the gastrointestinal tract. In this case, absorption appears to occur more rapidly when

EDC is administered in water compared to administration in oil (Withey *et al.* 1983). However, exposure via oil gavage is usually as a bolus dose, whereas drinking water exposure comes from many episodes spread out through the course of the day. In addition, an oil vehicle may alter the manner in which EDC is absorbed from the gastrointestinal tract. Lipids, such as those present in oil, are absorbed from the gastrointestinal tract via a pathway other than the hepatic portal system. Specifically, lipids are absorbed via chylomicrons to the lymphatic system, where they are transported directly to the venous circulation via the thoracic duct (effectively bypassing first-pass metabolism in the liver). It is conceivable that a significant fraction of the EDC dose administered in oil is absorbed along with the lipids in this manner, however, the extent to which this may occur is not known.

- *Metabolism* - It is possible that an oil vehicle can affect the metabolism of EDC via changes in cytochrome P-450 induction, reduced glutathione levels, or via changes in cellular levels of metabolic cofactors (*i.e.*, NADPH, NADH, or O_2). However, information regarding these potential impacts are not available.

- *Toxic Interaction* - Finally, it is also possible that a component of the oil interacts with EDC which in some way exacerbates toxicity.

The strong involvement of the thymus in the toxic action of EDC when administered in oil but not when administered in drinking water (NTP, 1991), suggests that the primary effect of the oil vehicle

may be on absorption. Under the assumption that a fraction of the EDC dose administered in oil is absorbed via lymphatic uptake, organs in the thoracic region such as the thymus could receive a large fraction of the bolus dose prior to distribution to the rest of the body. It is anticipated that the differences in absorption kinetics between water and oil may partially explain these differences in toxic response and these kinetic differences will be quantitatively accounted for in the PBPK modeling.

5.0 REFERENCES

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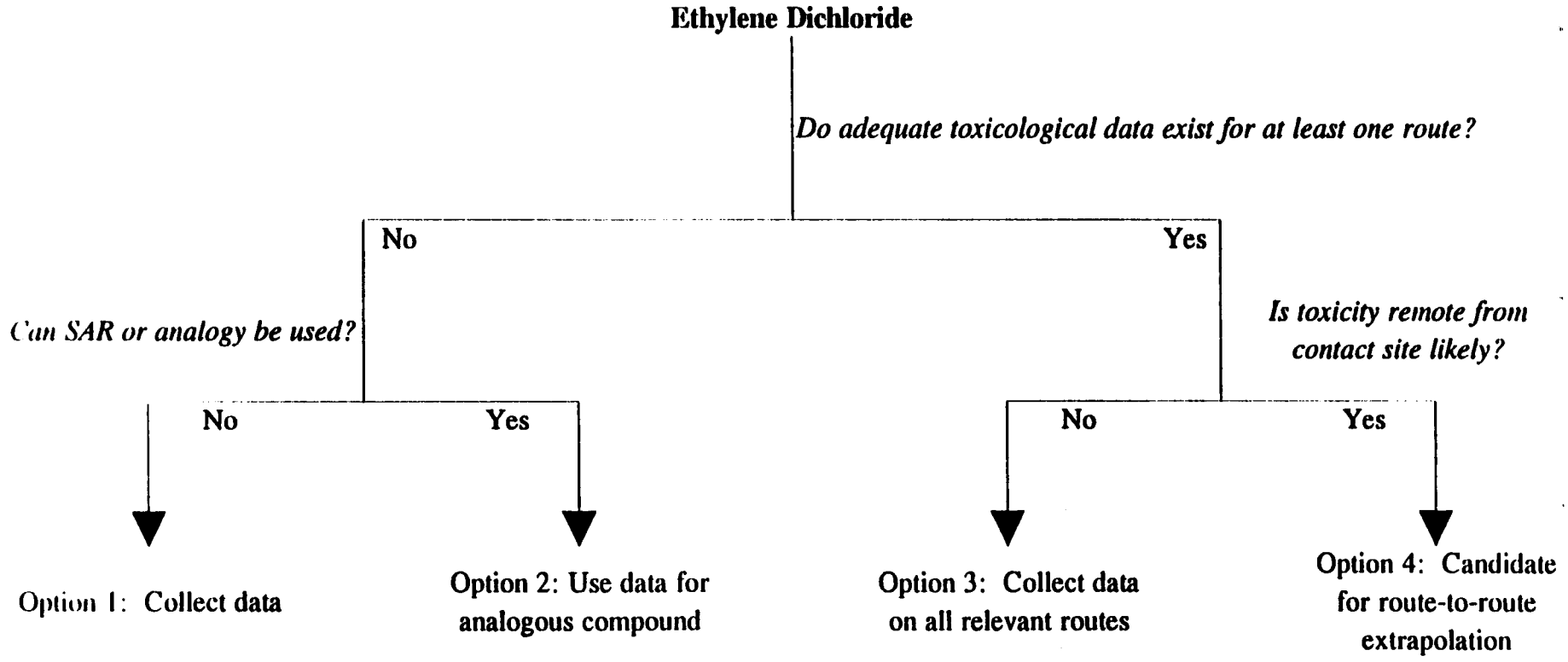
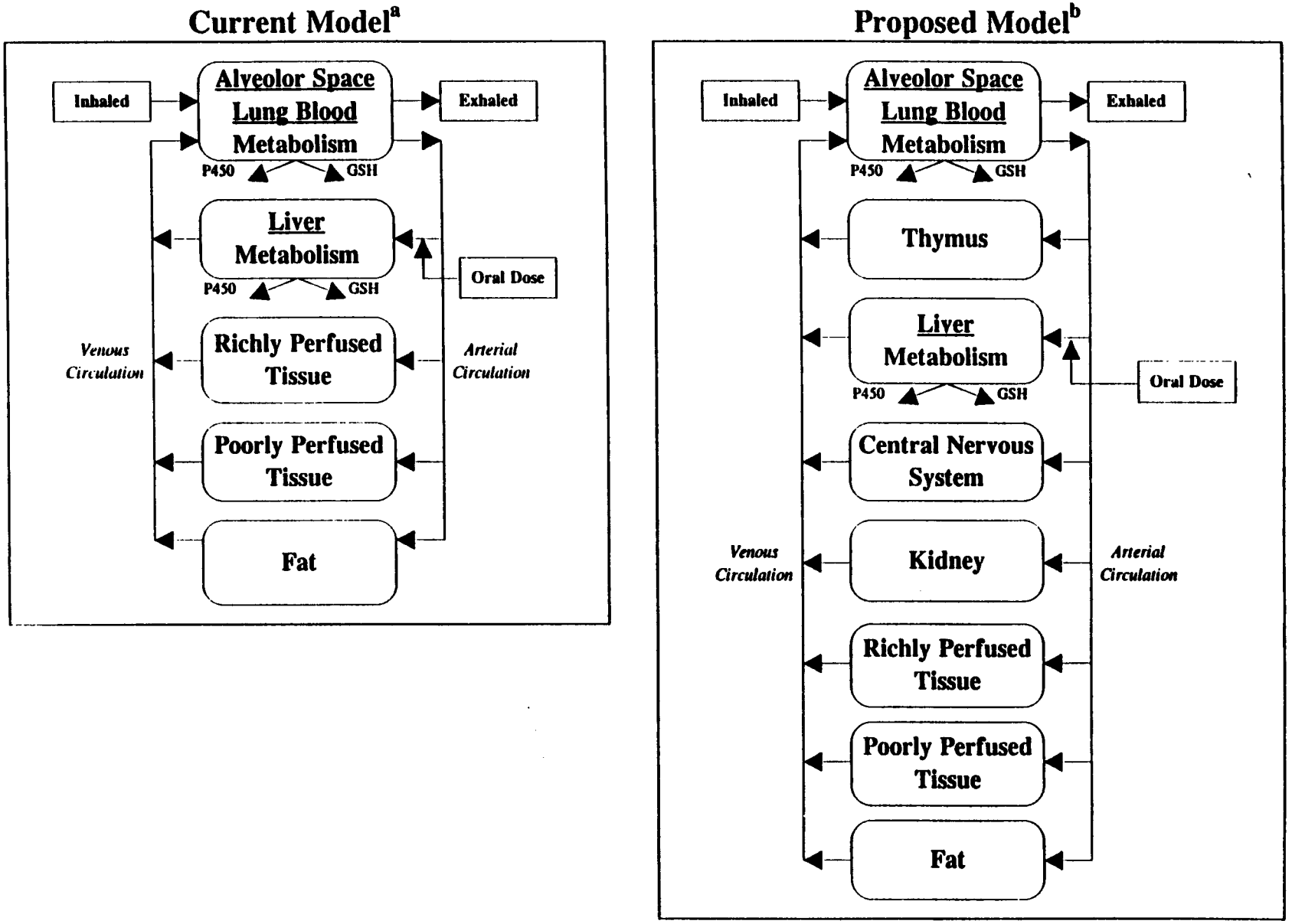
Figure 1
Decision Tree for Route-to-Route Extrapolation
Ethylene Dichloride*



*Adapted from Gerrity and Henry (1990)

Figure 2
Comparison of Current and Proposed PBPK Models for Ethylene Dichloride



[a]Adapted from D'Souza et al. (1987)

[b]The model could be expanded further for developmental and reproductive effects if USEPA does not feel that available inhalation studies are sufficient.

Table 1
Comparison of Candidate Studies to OPPTS Test Guidelines for Acute Toxicity

	Candidate Study for Route Extrapolation
Study:	Daniel et al. (1994)
Subchronic effects observed:	Lack of histopathological effects
LOAEL:	None
NOAEL:	100

Parameter	Recommended	
Test species	Rat or mouse	Rat
Strain	F344 or B6C3F1	Sprague-Dawley
Age	Young adult	Young adult
Sex	Both	Both
Health Status	Evaluate initially	Adequate
Number of animals	5 /sex/dose	10/sex/dose
Control Groups	Concurrent sham or vehicle control	Vehicle control
Concentration level and selection	> =4 (including control)	0, 10, 30, 100, 300 mg/kg-day
Limit dose	MTD or 5 mg/L	Adequate
1-hr study	If triggered, > =3	Not applicable
8-hr study	If triggered, > =3	Not applicable
Exposure	Nose only or whole body	Oral (oil gavage)
Environmental conditions	~22°C; 40 - 60% humidity	Adequate
Exposure periodicity	4 hr (1 & 8hr, if triggered)	1x/d, 10 d
Physical measurements	Environmental conditions monitored	Adequate
Observation period	24 hr	10 days
Gross pathology	Full necropsy, organ weights	Adequate
Histopathology	Histopathology, including respiratory tract	Adequate
Bronchoalveolar lavage	Provide indicators of lung damage	Not evaluated
Equipment and test methods reporting	Adequately defined	Adequate
Results reporting	Tabular results, per animal, statistics	Adequate

Table 2
Comparison of Candidate Studies to OPPTS Test Guidelines for Subchronic Toxicity

		Candidate Studies for Route Extrapolation			
	Study:	NTP (1991) - Drinking water study in rats	NTP (1991) - Corn oil gavage study in rats	NTP (1991) - Drinking water study in mice	Daniel et al. (1994)
	Subchronic effects observed:	Mild renal tubular regeneration, increased kidney	Necrosis of the thymus	Mild-to-moderate renal tubular regeneration	Liver and kidney weight changes, hematological effects
	LOAEL:	102	240	2710	75
	NOAEL:	58	120	781	37.5
Parameter	**Recommended**				
Test species	Rat (other mammal)	Rat	Rat	Mouse	Rat
Strain	Common lab	F344/N, Sprague-Dawley, Osborne Mendel	F344/N	B6C3F1	Sprague-Dawley
Age	Young, healthy	Young, healthy	Young, healthy	Young, healthy	Young, healthy
Sex	Both	Both	Both	Both	Both
Number of animals	10/sex/dose, more if interim sacrifice	10-20/sex/dose	10/sex/dose	10/sex/dose	10/sex/dose
Husbandry	Standard	Adequate	Adequate	Adequate	Adequate
Control Groups	Concurrent sham or vehicle control	Untreated	Vehicle	Untreated	Vehicle
Concentration level and selection	> =4 (including control)	0, 49-82, 86-126, 147-213, 259 428, 515-727 mg/kg-day	0, 30, 60, 120, 240, 480 mg/kg day (males); 0, 18, 37, 75, 150, 300 mg/kg-day (females)	0, 249, 448, 781, 2710, 4207 mg/kg-day (males); 0, 244, 647, 1182, 2478, 4926 mg/kg-day (females)	0, 37.5, 75, 150 mg/kg-day
Limit dose	Maximum tolerable dose achieved	Adequate	Adequate	Adequate	Adequate
Intermediate dose	Provides gradiation of effects	Adequate	Adequate	Adequate	Adequate
Lowest dose level	Should provide NOAEL	Adequate	Adequate	Adequate	Adequate
Administration of the substance	6 hr/day, 7 day/week	Daily	Daily	Daily	Daily
Observation period	90 days	13 weeks	13 weeks	13 weeks	90 days
Exposure specifications and physical measurements	Nose only or whole body	Oral (drinking water)	Oral (oil gavage)	Oral (drinking water)	Oral (oil gavage)
Observation of animals	Mortality (daily), clinical (weekly)	Adequate	Adequate	Adequate	Adequate
Clinical pathology	Hematology and clinical chemistry	Adequate	Adequate	Adequate	Adequate
Ophthalmological examinations	High-dose and control	Necropsy and histology of eyes	Necropsy and histology of eyes	Necropsy and histology of eyes	
Gross pathology	Gross necropsy and organ weights	Adequate	Adequate	Adequate	Adequate
Histopathology	Histopathology, including respiratory tract	Adequate	Adequate	Adequate	Adequate
Results reporting	Tabular results, statistics	Tabular means, standard deviations, incidences	Tabular means, standard deviations, incidences	Tabular means, standard deviations, incidences	
Evaluation	Adequately described	Adequate	Adequate	Adequate	

Table 3
Comparison of Studies to OPPTS Guidelines for Developmental Toxicity

		Existing Inhalation Studies			Candidate for Route Extrapolation
		Payan et al. (1995)	Rao et al. (1980)[a]	Rao et al. (1980)[a]	Lane et al. 1982
	Effect:	Maternal toxicity	Maternal toxicity	Maternal toxicity	None
	LOAEL:	329 ppm	300 ppm	300 ppm	None
	NOAEL:	254 ppm	100 ppm	100 ppm	50 mg/kg-day
Parameter	**Recommended**				
Test Species	>2	Rat	Rat	Rabbit	Mouse
Strain	Common laboratory	Sprague-Dawley	Sprague-Dawley	New Zealand White	Swiss
Sex	Pregnant female	Pregnant female	Pregnant female	Pregnant female	Pregnant female
Route	Inhalation	Inhalation	Inhalation	Inhalation	Oral (drinking water)
Number of animals	>20/group (rats); >12/group (rabbits)	26/group	16-30	19-21	10-18
Control group	Filtered air/vehicle	Filtered air	Filtered air	Filtered air	Untreated/Vehicle
Concentrations	>4 (including control)	0, 150, 194, 254, 329 ppm	0, 100, 300 ppm	0, 100, 300 ppm	0, 5, 15, 50 mg/kg-day
Dose Seletion	Characterizes dose response	Adequate	Significant toxicity at high dose	Adequate	No toxicity observed in dose range
Exposure Duration	6 hr/d	6 hr/d	7 hr/d	7 hr/d	Daily
Observation Period	Gd 6-15 (rat); Gd6-18 (rabbit)	Gd 6-20	Gd 6-20	Gd 6-18	Gd 1-21
EnvironmentalConditions	Standard/monitored	Adequate	Adequate	Adequate	Adequate
Observation	Daily	Adequate	Adequate	Adequate	Adequate
Gross Necropsy	Uterus/embryo	Adequate	Adequate	Adequate	Adequate
Data Reporting	Tabular/detailed	Adequate	Adequate	Adequate	Adequate
Evaluation	Statistics/described	Adequate	Adequate	Adequate	Adequate

[a]This study is also cited as Murray et al. (1980); Shell Oil (1979); Schlacter et al. (1979)

Table 4
Comparison of Candidate Studies to OPPTS Guidelines for Reproductive Toxicity

		Existing Inhalation Study	Candidate for Route Extrapolation
		Rao et al. 1980	Lane et al. 1982
	Effect:	None	None
	LOAEL:	None	None
	NOAEL:	150 ppm	50 mg/kg-day
Parameter	**Recommended**		
Species	Rat (other mammal)	Rat	Mouse
Strain	Common laboratory	Sprague-Dawley	Swiss
Age	Young adult	Adequate	Adequate
Number of animals	>20 pregnant females	20-30/group	10M 30F/group
Doses	>4 (including control)	0, 25, 75, 150 ppm	0, 5, 15, 50 mg/kg-day
Route	Inhalation	Inhalation	Oral (drinking water)
Exposure Frequency	7 d/wk	6 hr/d 5-7 d/wk	7 d/wk
Design	Multigenerational; exposure > 10 wk prior to mating	Single generation; F0 exposed 60 d prior to mating	Multigenerational; F1 exposed 11 wk prior to mating
Mating	Random	Random	Random
Control	Untreated/vehicle	Untreated	Untreated/vehicle
Observations (adult)	Daily/weekly	Daily	Weekly
Observations (litter)	PND 0, 4, 7, 14, 21	PND 1, 7, 14, 21	PND 0, 4, 7, 14, 21
Endpoints	Fertility index, gestation index, sperm morphology, age at vaginal opening or preputial separation, gross necropsy, organ weight, histopathology	Fertility index, gestation index, gross necropsy, terata, limited histopathology	Fertility index, gestation index, gross necropsy, terata, dominant lethal mutation
Data reporting	Tabular	Adequate	Adequate
Evaluation	Statistics described	Adequate	Adequate

Table 5
Comparison of Candidate Studies to OPPTS Test Guidelines for Neurotoxicity

		Candidate Studies for Route Extrapolation	
	Study	NTP 1991 (drinking water study)	NTP 1991 (oil gavage study)
	Neurological Effects Observed	None	Tremors, necrosis of the cerebellum
	LOAEL	NA	240 mg/kg-day
	NOAEL	492 mg/kg-day	120 mg/kg-day
Parameter	**Recommended**		
Test species	Rat (mice or dog)	Rat	Rat
Strain	Common	F344/N, Sprague-Dawley, Osborne Mendel	F344/N
Age	Young adult (>42 d)	42 d	42 d
Sex	Both	Both	Both
Number of animals	10/sex/dose	10-20/sex/dose	10-20/sex/dose
Control Groups	Concurrent sham or vehicle control	Vehicle control	Vehicle control
Concentration level and selection	>=4 (including control)	0, 49-82, 86-126, 147-213, 259-428, 515-727 mg/kg-day	Male (0, 30, 60, 120, 240, or 480 mg/kg-day); Female (0, 18, 37, 75, 150, or 300 mg/kg-day)
Dose selection (acute)	MTD; < 2g/kg	NA	NA
Dose selection (subchronic)	MTD; < 1 g/kg	Adequate	Adequate
Administration of the substan	Appropriate route of exposure	Oral	Oral
Combined protocol	Combine with other endpoints	NA	NA
Time of testing	Acute (0, 8hr, 7d, 14 d); subchronic (0, 4 wk, 8 wk, 13 wk)	90 days	90 days
Functional observational batt	Standard evaluations for appearance, behavior, and functional integrity	Clinical signs evaluated weekly	Clinical signs evaluated weekly
List of measures	Autonomic function, abnormal motor movements, response to general and sensory stimuli, alertness, grip strength, landing foot splay, body weight, behavioral changes	Abnormal body movements (tremor), body weight	Abnormal body movements (tremor), body weight
Motor activity	Individually assessed, automated	Not evaluated	Not evaluated
Neuropathology	Neuropathological examinations	Brain, sciatic (if signs present)	Brain, sciatic (if signs present)
Results	Tabular, per animal, statistics	Incidence, mean & SD, Dunn's or Shirley's tests	Incidence, mean & SD, Dunn's or Shirley's tests
Evaluation	Adequately described	Adequate	Adequate

Table 6
Summary of Pharmacokinetic Modeling Activities Proposed for Ethylene Dichloride

	Data Gap						
	Acute	Subchronic				Neurotoxicity	
Study	Daniel et al. (1994)	NTP (1991)	NTP (1991)	NTP (1991)	Daniel et al. (1994)	NTP (1991)	NTP (1991)
Species	Rat	Rat	Rat	Mouse	Rat	Rat	Rat
Route	Oral (oil gavage)	Oral (drinking water)	Oral (oil gavage)	Oral (drinking water)	Oral (oil gavage)	Oral (drinking water)	Oral (oil gavage)
Endpoint	Lack of histopathological effects	Mild renal tubular regeneration, increased kidney weight	Necrosis of the thymus	Mild-to-moderate renal tubular regeneration	Liver and kidney weight changes, hematological effects	None	Necrosis of the brain, clinical signs
LOAEL	None	102 mg/kg-day	240 mg/kg-day	2710 mg/kg-day	75 mg/kg-day	102 mg/kg-day	240 mg/kg-day
NOAEL	100 mg/kg-day	58 mg/kg-day	120 mg/kg-day	781 mg/kg-day	37.5 mg/kg-day	58 mg/kg-day	120 mg/kg-day
Dose Metric	Total metabolized (glutathione)	Total metabolized (glutathione)	Total metabolized (glutathione)	Total metabolized (glutathione)	Total metabolized (glutathione)	EDC and/or total metabolized (glutathione)	EDC and/or total metabolized (glutathione)
Target Tissue	Liver/kidney	Kidney	Thymus	Kidney	Liver/kidney	Kidney	Central nervous system